Allowable Claims

The Official Action dated October 19, 2005 indicates that claims 30-32, 41 and 43 are allowed and claims 8 and 14-29 are allowable if re-written in independent form to include all of the limitations of the base claim and any intervening claims.

During a telephonic interview on 11/29/05, the Examiner questioned the enablement for claims 30-32, 41 and 43.

In order to expedite issuance, Applicants propose canceling claims 30-32, 41 and 43 and to pursue these claims and the remaining rejected claims in a continuation patent application.

Accordingly, Applicants request that the following claims be allowed:

8. (Re-written in independent format) A computer implemented method of localizing a biomarker within a cell, comprising:

identifying portions of a first image of the cell that corresponds to a first defined area;

reducing representation of out-of-focus elements in the first image by manipulating the image pixel intensities of the first image based on image pixel intensities of a third image featuring a different depth of focus;

identifying portions of a second image of the cell that corresponds to at least one biomarker;

superimposing portions of the second image against portions of the first image to identify whether the biomarker is localized within the defined area.

9. (Amended) The method of claim 8 [1], wherein the defined area is selected from the group consisting of the cell nucleus, cytoplasm, nuclear membrane, cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.

10. (Amended) The method of claim 8 [1], wherein the biomarker is selected from the group consisting of a protein, peptide, nucleic acid, lipid or carbohydrate.

14. (Re-written in independent format) A method of localizing a biomarker within a sub-cellular compartment of a cell, comprising:

obtaining a first image of a cell treated with a first stain that is selective for a first sub-cellular compartment within the cell, a second stain that is selective for at least one biomarker and a third stain that is selective for a second defined area within the cell;

determining an intensity value for the first stain at a plurality of pixel locations in the first image;

based on the intensity values, determining pixel locations in the first image that correspond to the first sub-cellular compartment within the cell and assigning those pixel locations to the first sub-cellular compartment;

obtaining a second image of the cell and determining an intensity value for the second stain at a plurality of pixel locations in the second image;

comparing the first and second images, wherein the presence of pixel locations in the second image that are within the first sub-cellular compartment indicate that the biomarker is within the first sub-cellular compartment;

obtaining a third image of the cell and determining an intensity value for each pixel location of a plurality of pixel locations in the third image;

based on the intensity values, determining which pixel locations in the third image correspond to the second defined area of the cell and assigning those pixel locations to the second defined area; and identifying which of the pixel locations in the second image are within the second defined area.

15. (Cancelled)

16. The method of claim 15, wherein the first, second, and third defined areas are selected from the group consisting of: a nucleus, cytoplasm, nuclear membrane, cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.

17. The method of claim 14, wherein the cell is contacted with a fourth stain that is selective for a defined area in the cell, and at least one pixelated image of the distribution of the fourth stain is acquired, the method further comprising reading a third intensity value for each of a plurality of pixels in the image of the fourth stain distribution;

determining a threshold intensity value from the third intensity values;

comparing the third intensity value for each of the plurality of pixels to the threshold intensity; and

assigning pixel locations to a mask based on the threshold intensity value.

18. The method of claim 17, wherein the pixel locations in the plurality of pixels in the image of the first stain distribution are the pixel locations in the mask set.

19. The method of claim 18, wherein the pixel locations assigned to the mask comprise the location of pixels having third intensity values equal to or greater than the threshold intensity value.

20. The method of claim 19, further comprising binning the third intensity values for each of the plurality of pixels in the image of the fourth stain distribution.

21. The method of claim 20, wherein the threshold intensity value is determined from an intensity value of a largest bin.

22. The method of claim 20, wherein the threshold intensity value is determined from an intensity value of a second largest bin.

23. The method of claim 17, further comprising comparing for each pixel location the first intensity value to the second intensity value and assigning the pixel location to the second defined area when the second intensity value is greater than the first intensity value.

24. The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values to determine a total signal intensity.

25. The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the first defined area in the cell.

26. The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values.

27. The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the second defined area.

28. The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values to determine a total intensity.

29. The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the third defined area.

44. (New) The method of claim 8, wherein the cells is in a tissue.

45. (New) The method of claim 8, wherein the cell has been fixed.